mezlocillin working standard with sufficient distilled water to obtain a concentration of 2.0 milligrams of mezlocillin per milliliter.

(*c*) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration; also, if packaged for dispensing, reconstitute as directed in the labeling using distilled water in lieu of the reconstituting fluid. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to a concentration of 2.0 milligrams of mezlocillin per milliliter (estimated).

(*d*) *Calculations*—(*1*) Calculate the mezlocillin content in micrograms per milligram as follows:

$$\text{Micrograms of mezlocillin per milligram of sample} = \frac{A_u \times P_a}{A_s \times W_u}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
W_u=Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the mezlocillin content of the single-dose vial as follows:

$$\text{Milligrams of mezlocillin per single-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
d=Dilution factor of the sample.

(*3*) Calculate the mezlocillin content of the multiple-dose vial as follows:

$$\text{Milligrams of mezlocillin per multiple-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
d=Dilution factor of the sample;
n=Volume of sample solution assayed.

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of mezlocillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of mezlocillin per milliliter.

(7) *Specific rotation.* Dilute an accurately weighed sample with sufficient distilled water to obtain a concentration of approximately 10 milligrams of mezlocillin per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube.

(8) *Identity.* Proceed as directed in § 436.311 of this chapter, diluting the sample with distilled water to a concentration of 4 milligrams of mezlocillin per milliliter, except:

(i) Use the mezlocillin working standard and dilute with distilled water to a concentration of 4 milligrams of mezlocillin per milligram;

(ii) In lieu of the ninhydrin spray solution, after the plate is dried with a current of warm air, expose the plate to iodine vapors for about 30 seconds; and

(iii) Mezlocillin has an R_f value of about 0.67.

[46 FR 58298, Dec. 1, 1981, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.41 Nafcillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Nafcillin sodium monohydrate is the monohydrated sodium salt of 6-(2-ethoxy-1-naphthamido) penicillanic acid. It is so purified and dried that:

(i) It contains not less than 820 micrograms of nafcillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.5 percent and not more than 5.3 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 5.0 and not more than 7.0.

(v) It is crystalline.

(vi) Its nafcillin content is not less than 82.0 percent.

(vii) It gives a positive identity test for nafcillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods: however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2 micrograms of nafcillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(b) of this chapter.

(6) *Nafcillin content.* Dissolve an accurately weighed portion of the sample in a sufficient accurately measured volume of distilled water to obtain a concentration of 0.05 milligram of nafcillin per milliliter (estimated). Treat a portion of the nafcillin working standard in the same manner. Using a suitable spectrophotometer equipped with quartz cells and distilled water as a blank, scan the absorption spectra of the sample and the nafcillin working standard solutions between the wavelengths of 245 nanometers and 340 nanometers. Determine the absorbance of the sample and working standard solutions at the absorption maximum at 280±3 nanometers. (The exact position of the maximum should be determined for the particular instrument used.) Calculate as follows:

$$\text{Percent nafcillin} = \frac{\text{Absorbance of sample} \times \text{weight in milligrams of standard} \times \text{volume of sample solution} \times \text{nafcillin content of standard in percent}}{\text{Absorbance of standard} \times \text{weight in milligrams of sample} \times \text{volume of standard solution}}$$

(7) *Identity.* The absorption spectrum of the sample determined as directed in paragraph (b)(6) of this section compares qualitatively with that of the nafcillin working standard.

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